Study of HSA interactions with arachidonic acid using spectroscopic methods revealing molecular dynamics of HSA-AA interactions

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Abstract. The interaction between human serum albumin (HSA) and arachidonic acid (AA) as an unsaturated fatty acid were investigated in the present study using methods including UV-VIS spectrophotometry, fluorescence and circular dichroism (CD) spectroscopy, lifetime measurements, fluorescence anisotropy measurements and visual molecular dynamics (MD). The thermodynamic parameters were assessed from HSA thermal and chemical denaturation in the presence and absence of AA. From the thermal denaturation, the T_m and $\Delta G^{\circ}_{(298K)}$ magnitudes obtained were 327.7 K and 88 kJ/mol, respectively, for HSA alone, and 323.4 K and 85 kJ/mol, respectively, following treatment with a 10 μM AA concentration. The same manner of reduction in Gibbs free energy as a criterion of protein stability was achieved during chemical denaturation by urea in the presence of AA. The present study investigates HSA binding nature through MD approaches, and the results indicated that the binding affinity of AA to the subdomain IIA of HSA is greater compared with that of subdomain IIIA. Although the HSA regular secondary structure evaluation by CD exhibited a minor change following incubation with AA, its tertiary structure revealed an observable fluctuation. Thus, it appears that the interaction between AA and HSA requires minor instability and partial structural changes.

Introduction

Albumin is the most abundant plasma protein (35-50 g/l human serum) with a molecular weight of 66.5 kDa. The functions and binding properties of human serum albumin (HSA) are multifold (1). HSA facilitates the transport and disposition of various endogenous and exogenous components to their specified targets. Albumin is emerging as a versatile protein carrier for ligand targeting and also for peptide pharmacokinetic profile improvement or for protein-based ligands (2). An X-ray crystallographic study reveal that the heart shaped HSA consists of three structurally similar domains (I, II and III), each of which contains two subdomains (A and B) (3). It has been suggested that the principal regions of the ligand which bind to HSA are located in hydrophobic cavities in subdomains IIA and IIIA, which respectively are designated as sites I and II (4,5). The binding affinity offered by site I is mainly through hydrophobic interactions, whilst site II involves a combination of hydrophobic, hydrogen bonding and electrostatic interactions (6). The unique nature of the HSA ligand binding properties reflect its multi domain organization, and it is also one of the most important structure-function associations ever reported for monomeric proteins (7). HSA is known to be able to carry almost every small molecule; thus, it may be a potential contender for being a cargo molecule/or nano-vehicle for clinical, biophysical and industrial purposes (8,9). This protein serves an essential function as a transporter of various unsaturated fatty acids including arachidonic acid (AA) and hormones including L-thyroxin to the target sites (10). Polyunsaturated fatty acids (PUFAs) are a group of fatty acids which contain more than one double bond in their backbone. This class comprises a number of important compounds, including essential fatty acids (11). AA is one of the most important biological lipids which is present in most organic species, is a physiologically significant omega-6 fatty acid and also the precursor of prostaglandins and other active molecules (12). Human are able to easily metabolize linoleic acid to form n-6 PUFA. It has been suggested that AA functions as a protective agent against melanoma cancer and blood coagulation (13). Furthermore, lipid mediators generated from long-chain PUFA (AA in the n-6 series and eicosapentaenoic acid and docosahexaenoic acid in the n-3 series) have important functions in immune regulation and inflammation (14,15).

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